REMARKS

Claims 1-9 are currently under examination in the Application. Upon entry of this amendment claims 1-9 are hereby canceled and new claims 10-26 are added. The claims closely track the previous claims, but have been altered to focus on one aspect of the present invention. Support for claim 10 can be found for example at page 7, lines 13-17; support for the molecular weight limitation of 890,000 Da for hyaluronan, can be found in the previous claims as well as at, for example, page 29, line 28 and page 17, line 37; support for the polydispersity of 1.78 can be found in, for example, Figure 6. It is well known to the person skilled in the art that the following formula is used to determine polydispersity:

Kav=Ve-V0/Vt-Vo

After determining the modal Kav, the polydispersity is calculated by multiplying the modal Kav by 2, and this figure is then added to 1. As seen in Figure 6, the hyaluronan is eluted at a modal Kav of 0.39, meaning that half of the 890,000 Da hyaluronan molecular weights are eluted within the first 40% of the gel and the second range of molecular weights is in the next 40% of the gel bed. The polydispersity of the 890,000 Da hyaluronan is therefore calculated at 1.78. The remaining claim limitations find support in the previously pending claims. No new matter has been added. Reconsideration is respectfully requested in view of the following remarks.

Continued Examination under 37 C.F.R. § 1.114

Applicants thank the Examiner for noting that present application was eligible for continued examination under 37 CFR 1.114 and that the finality of the previous Office Action has been withdrawn.

Rejection Under 35 U.S.C. § 112 (NEW MATTER)

Claims 1-9 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. In particular, the Action contends that the recitation of hyaluronic acid (HA) having molecular weight of greater than or equal to 750,000 Daltons is new matter.

Applicants respectfully traverse the rejection and submit that HA of various weights are described throughout the specification. In particular, support for HA greater than 750 kDa can be found, for example, at page 17, line 37 and page 29, line 28. HA of 750 kDa is explicitly recited at page 39, line 31 and page 40, line 16. Further, 750 kDa is used in Example 13 which describes the use of a formulation comprising HA and 5-Fluorouracil. This description of HA having a molecular weight of 750 kDa can be found at page 79, line 35, at page 80, line 4, and at page 81, Table 4. As to the assertion by the Examiner that there is no specific support for greater than 750 kDa, Applicants submit that Applicants need not explicitly recite embodiments that are clearly contemplated and within the appreciation of those of ordinary skill in the art upon reviewing the disclosure. The courts, and specifically the U.S. Court of Appeals, Federal Circuit, has ruled that 35 U.S.C. § 112 does not require that the specification contain the literal language (*ipsis verbis*) of an amendment to the claims. *See, e.g.*, In re Wright 866 F.2d 422 (Fed. Cir. 1989), as long as one of skill in the art would appreciate the disclosure to contain such teachings. In the present case, one of skill in the art would recognize that given the teachings and data provided herein that use of HA greater than or equal to 750 kDa has certain advantages. Nevertheless, without acquiescing to the grounds of rejection and solely in order to facilitate prosecution, Applicants have submitted to claims that refer to specific molecular weights that the Examiner has previously noted support for. Accordingly, Applicants submit that the claims are fully supported by the specification and do not contain new matter. Reconsideration and withdrawal of this rejection are respectfully requested.

Rejection Under 35 U.S.C. § 103(a)

Claims 1-9 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of Falk *et al.*, (U.S. Patent No. 5,985,850). In particular, the Action alleges that Falk *et al.* disclose injectable formulations comprising anti-cancer agents or chemotherapeutic agents combined with HA wherein the HA has a preferred molecular weight of less than 750 kDa. Further, the Action contends that Applicants' Declaration under 37 C.F.R. § 1.132 was not commensurate in scope with the claims in that no data was provided at a molecular weight of 750kDa, and that the 30kDa data is much lower than 750kDa. The Examiner also asserts that since the claims have no upper limit on HA weight that a single data point of 890 kDa is insufficient to support the claim scope and demonstrate surprising results over the art. Claims 1-9 also stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Turley *et al.* (U.S. Patent No. 6,475,795) in view of Sola *et al.* (U.S. Patent No. 6,214,860). Specifically, the Action contends that Turley *et al.* disclose pharmaceutical compositions that comprise anti-sense nucleic acid bound to hyaluronic acid having a molecular weight of between 150 kDa and 750 kDa for treating diseases or conditions treatable using gene therapy. Turley *et al.* also allegedly disclose using hyaluronan having molecular weights of between 500 kDa and 800 kDa. The Action goes on to state that, while Turley *et al.* do not disclose non-polynucleic acid cytotoxic agents, "one cytotoxic agent can be used in place of another with the expectation of producing antineoplastic effect." Sola *et al.* generally teach cytotoxic agents such as paclitaxel, cisplatin, and camptothecin. As such, the Action asserts that it would have been obvious to the skilled artisan to combine the teachings of Turley *et al.* with the teaching of Sola *et al.* to arrive at Applicants' invention. Lastly, the Examiner asserts that even though HA was only used as a targeting agent that its activity in cancer would have been an inherent property.

Applicants respectfully traverse the rejection on the following grounds.

The present invention is directed to overcoming resistance of a cancer to an anti-cancer drug by administering the drug with HA. The treatment of a wide range of cancers with conventional therapeutic agents, such as methotrexate and irinotecan, is frequently rendered ineffective due to the resistance of the cancer to the agents. There are two types of resistance,

acquired resistance and inherent resistance. In the case of acquired resistance, repeated exposure of a cancer cell to a specific drug can stimulate the cell to form several survival mechanisms to overcome drug sensitivity. Such changes are believed to include increased expression of p-glycoprotein efflux pump and large hyaluronan glycolax. In the case of inherent resistance, some tumor cells innately contain a high expression of proteins such as p-glycoprotein that can act as an efflux pump for the anti-cancer agent.

Assuming, *arguendo*, that the references cited by the Examiner disclose the use of HA in combination with anti-cancer agents to increase the efficacy of the anti-cancer agent, they clearly do not disclose that either alone or in combination with other agents the desirability of or utility of delivery of HA with a drug to drug resistance cancers. For example, Falk *et al.* merely teach the use of HA to facilitate an anti-cancer agent's penetration through the tissue (including scar tissue) across the cell membranes into the individual cells to be treated, thereby allegedly enhancing the efficacy of the anti-cancer agent. Nowhere do *Falk et al.* teach or suggest that the administration of HA with an anti-cancer agent can reverse or overcome the resistance of the cancer cells to the anti-cancer agent. Such a reversal or overcoming resistance is an entirely different mechanism and lends itself to far different treatments when compared to an agent that enhances cellular penetration.

Claim 10, is directed to a method for enhancing efficacy of a drug for a cancer cell by co-administering the HA with the drug to which the cancer cell is resistant. Nowhere do Falk *et al.* teach or suggest that the use of HA in combination with an anti-cancer agent is capable of overcoming drug resistance. Accordingly, Applicants respectfully submit that the present invention is not obvious in light of Falk *et al.*

With respect to the rejections in light of Turley *et al.* in view of Sola *et al.* we respectfully disagree for the reasons noted above, but also due to the fact that the combination of the references is clearly not one that the skilled artisan would contemplate (gene therapy combined with non-genetic cytotoxic agents).

Turley *et al.* only teach the use of HA as a targeting agent for gene therapy. Turley *et al.* provide no data, suggestion or teaching that the use of HA when combined with an anti-cancer agent would reverse or overcome the resistance of the cancer cells to the anti-cancer

agent. The teachings of Sola *et al.* do not overcome the deficiencies of Turley *et al.* in that Sola *et al.* merely provide general teachings on cytotoxic agents with no teaching or suggestion whatsoever of the use of HA.

As discussed above, the claims are directed to a method for enhancing efficacy of a drug for a cancer cell by co-administering the HA with the drug to which the cancer cell is resistant. Turley *et al.* and Sola *et al.* either alone or in combination, simply do NOT teach methods of treating drug resistance using HA. Accordingly, we submit that the present invention is inventive in light of Turley *et al.* when combined with Sola *et al.*

In order to further demonstrate to the Examiner that HA is surprisingly effective in reducing or overcoming acquired and inherent resistance of cancers to anti-cancer agents, we provide herewith additional Examples 1 to 4. If the Examiner believes it to be helpful the following examples can be set forth in a 1.132 Declaration to demonstrate the objective non-obviousness of the present approach.

In Example 1 (attached), a human colon cancer tumor was maintained in mice which were treated either with irinotecan alone or irinotecan in combination with HA. For the first 9 weeks of the experiment, the tumors of both groups of test animals reduced in volume. However, thereafter it can readily be seen that while the group of test animals that was being administered irinotecan in combination with HA continues to show a reduction in tumor volume over weeks 10 to 15, in the group that was being administered irinotecan alone tumor volume steadily increased such that by week 15 had returned to its original size. This clearly demonstrates that the cancer cells developed an acquired resistance to the irinotecan and that this acquired resistance was avoided when HA was also administered.

In Example 2 (attached), a breast cancer cell line that does not respond to methotrexate was treated with varying concentrations of ether methotrexate alone or the same concentration of methotrexate in combination with HA. It will be readily appreciated that when the methotrexate was used alone, the cell population showed no reduction in number, thus demonstrating a clear inherent resistance to the methotrexate. In contrast, when HA was administered with the methotrexate there was a marked reduction in the cell population at all methotrexate concentrations, thus demonstrating that this inherent resistance had been overcome.

In Example 3 (attached), a colon cancer cell line (LIM 1215) which does not respond to methotrexate was treated with varying concentrations of either methotrexate alone or with HA. First, it will be readily appreciated that there is no reduction in the cell population at any of the methotrexate concentrations when used alone, thus demonstrating clear inherent resistance to the methotrexate. Second, it can be seen that when the methotrexate is combined with HA, there was a significant drop in cell numbers.

In Example 4 (attached), tests were performed to determine whether HA could be used with a wide variety and several classifications of cytotoxic agents to overcome, reduce or prevent drug resistance of a cancer cell line. The results demonstrate very clearly that where inherent resistance was demonstrated against the agent alone (*e.g.*, for 5-Fu or gemcitabine), the co-administration of HA reduced this significantly. Furthermore, where the cell line initially responded to the cytotoxic agent but then became resistant to it (*i.e.*, acquired resistance- see irinotecan or doxorubicin), co-administration with HA resulted in a remarkable reduction in the acquired resistance.

Clearly given the previous use of HA to increase cellular penetration of a drug, one would not be motivated to overcome resistance to a drug by delivering HA in combination therewith. Given the newly submitted claims and the comments above, Applicants submit that the claimed invention is clearly not obvious in view of Falk *et al.* or over Turley *et al.* in view of Sola *et al.* Reconsideration and withdrawal of these rejections is respectfully requested.

The claims are now believed to be in condition for allowance. A good faith effort has been made to place the application in condition for allowance. However, should any further issue require attention prior to allowance, the Examiner is requested to contact the undersigned at 206-622-4900 to resolve same.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

/William Christiansen/
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WTC:jto

Enclosure:
Examples 1-4

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961575_1.DOC

Additional Example 1

When a cancer cell is repeatedly exposed to a specific drug it can form several survival mechanisms to overcome this drug sensitivity. In this example human colon cancer tumours maintained in immune deficient mice was treated either by co-administering hyaluronan having a Mr of 800-900 kDa and irinotecan (HyCAMP) or by administering the same concentration of irinotecan (Camptosar®) alone. The percentage change in tumour volume was measured over time. It is evident from Figure 1 below that initially the human colon tumour responded to both drug regimens (there is a decrease in tumour volume) during weeks 1-9 of treatment. However, during this time a sub-population of the tumour cells developed a resistance to the Camptosar® (irinotecan alone), as can be seen from weeks 10-15 when the tumour volume increases thereby indicating the tumour has acquired resistance to the drug. In contrast, in tumours treated with irinotecan formulated with hyaluronan (HyCAMP) the acquired resistance is circumvented as the tumours continue to respond to the drug.

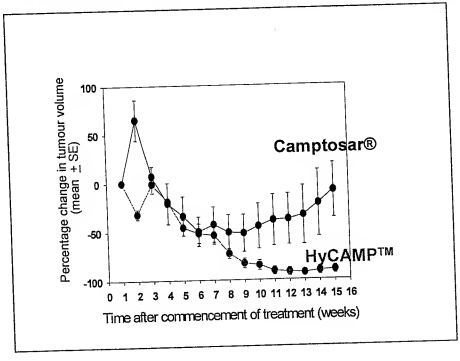


Figure 1

Additional Example 2

Some tumour cells innately contain high expression of proteins such as p-glycoprotein which can act as an efflux pump for drugs, and therefore these cells are considered inherently resistant to specific drugs as this characteristic is present before exposure to said drug. To demonstrate that hyaluronan can overcome this inherent drug resistance, a cell line which does not respond to methotrexate (ZR-75-1) was treated with methotrexate alone and a combination of methotrexate and hylauronan having a modal Mr of 750,000 and a polydispersity of 1.6 at varying concentrations of methotrexate. It can be clearly seen from Figure 2 below that while the cell line was resistant to methotrexate alone at all concentrations, when hyaluronan was used in combination with the methotrexate there was a marked reduction in tumour volume. This provides clear evidence of the ability of hyaluronan to reduce the inherent resistance of some cancer cell lines to specific cancer drugs.

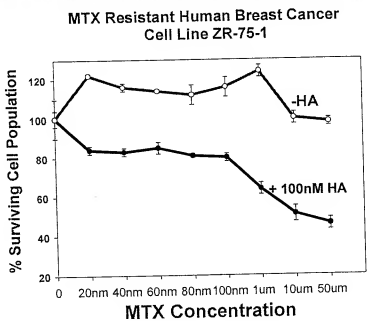


Figure 2

Additional Example 3

In the present experiment, a colon cancer cell line (LIM 1215) was tested for its susceptibility to varying concentrations of methotrexate with low (35 KDa, 220 kDa, 420 kDa) and high (750 kDa, 880 KDa and 1429 KDa) molecular weight hyaluronan at a concentration 86μg/ml as well as to varying concentrations of methotrexate alone. After 3 days, the various preparations were compared for their anti-cancer properties, based on the percentage reduction of the tumour volume compared to the control where no drug was administered. It is immediately apparent from Figure 3 below that the LIM 1215 cell line is resistant to methotrexate in the absence of hyaluronan. However, when combined with hyaluronan the resistance is overcome, and higher molecular weight hyaluronans (>420 kDa) have the best effect, particularly at a molecular weights of ≥750 kDa.

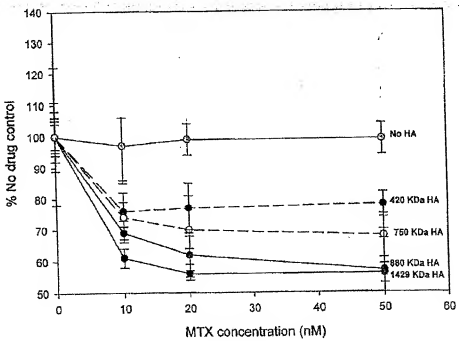


Figure 3

Additional Example 4

In this example, we set out to demonstrate whether the formulation of hyaluronan (HA) with a wide variety and several classifications of cytotoxic agents could overcome or prevent drug resistance.

Methodology:

Test Articles

The following test articles and dosages were used in obtaining these *in vitro* and/or *in vivo* data:

Chemotherapeutic Drug	Classification of Chemotherapeutic Drug	Modal Molecular Weight of the HA Combined with Chemotherapeutic Drug (kilo Dalton)
Methotrexate	Anti-metabolite	750
5-Fluorouracil	Anti-metabolite	750
Cyclophosphamide	Alkylating agent	750
Doxorubicin	Antibiotic	750
Irinotecan	Topopisomerase inhibitor	800-900
Oxaliplatin	Platinum	800-900
Gemcitabine	Anti-metabolite	800-900

Human Tumour Model

Athymic CBA/WEHI nude female mice (Walter and Eliza Hall Research Institute, Melbourne, Australia) were maintained under specific pathogen-free conditions, with sterilised food and water available *ad libitum*. Human tumours were generated in each mouse by growing the cancer-specific cell line as per suppliers instructions and injecting 1 x 10^7cells into the mammary fat pad directly under the first nipple. Treatment with the chemotherapeutic drug when formulated with and without hyaluronan commenced approximately 4-8 weeks after the cancer cell inoculation when the tumour volume reached <0.40% of net body mass (50-100mm^3).

Administration of drugs and control vehicles

To demonstrate that HA when formulated with chemotherapeutic drugs consistently resulted in overcoming of drug resistance, a wide selection and classification of chemotherapeutic drugs were tested:

Chemotherapeutic Drug	Classification of Chemo-therapeutic Drug	Modal Molecular Weight of the HA Combined with Chemo-therapeutic Drug (kilo Dalton)	Type of cancer treated	Dose of chemo-therapeutic drug (mg/kg/ week)	Dose of HA (mg/kg/ week)
Methotrexate	Anti-metabolite	750	Breast	15	12.5
5-Fluorouracil	Anti-metabolite	750	Breast	30	12.5 & 13.3
Doxorubicin	Antibiotic	750	Breast	6, 9 12 & 16	12.5
Irinotecan	Topoisomerase inhibitor	800-900	Colon	50, 100, 150 &200	13.3, 26.6, 75 &150
Oxaliplatin	Platinum	800-900	Colon	8	75 and 150
Gemcitabine	Anti-metabolite	800-900	Pancreatic	510	450

Treatments were quantitatively administered via the tail vein. The injection syringe was weighed before and after injection and the weights recorded on the Injection Mass Data Sheet. The dose administered was calculated using the following formulas:

volume injected (ml) = mass of syringe before injection (g) - mass of syringe after injection (g)

concentration in injection solution (mg/ml) = stock solution solution concentration X dilution factor

Mass injected (mg) = concentration in injection solution (mg/ml) X volume injected (ml)

$$\text{Dose administered (mg/kg)} = \frac{\text{mass injected (mg) X 1000}}{\text{mouse mass (g)}}$$

Definition of experimental end-point

The experimental end-point used for these studies were determined either by the disease progression in the animals or when the treatment regimen was completed. The following criteria were used to determine if an animal has reached the stage of experimental end-point of necessary death

i) tumour mass is so large the animal is immobilised
ii) animal is not eating or drinking and has experienced dramatic weight loss
iii) animal energy level at <1+
iv) tumour size is greater than 10% of body mass
v) Completion of treatment period

Monitoring of body mass, tumour volume and animal well being

Upon commencement of treatment, animal observations were made on a daily basis, including the day of experimental end-point. Animals were weighed, tumour volumes measured and animal well being monitored by noting energy levels, food intake and evidence of GI tract toxicity such as diarrhoea. Weight loss was monitored by calculating net body weight as estimated by subtracting tumour weight, which was calculated as 1g x tumour volume (cm^3). For demonstration of any weight changes the animal body weight was normalised to the body weight at the time of treatment commencement as:

$$\frac{\text{Body mass (ex tumour)– Body mass at commencement of treatment (ex tumour) X 100}}{\text{Body mass at commencement of treatment (ex tumour)}}$$

Analysis of Data

Comparison of treatment and control group data was achieved by statistical analysis using parametric t-test analysis. On failing of normal distribution, implementation of non-parametric analysis will be carried out using:

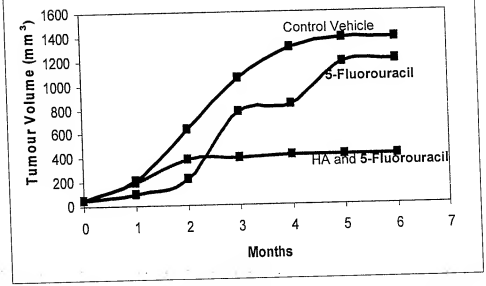
i) Mann-Whitney Rank Sum

ii) One-way Anova

Results

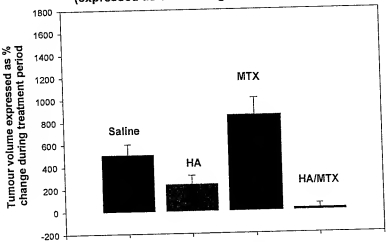
As seen in the graphic representations below it is evident that hyaluronan reduced the resistance of the tested cancer to a range of chemotherapeutic drugs that belonged to several classifications of cytotoxicty. In each case, the cell line demonstrated either inherent resistance to the administered drug alone (e.g. see 5-fluorouracil or gemcitabine) or acquired resistance to the administered drug alone (e.g. irinotecan or doxorubicin). Administration of the same drugs with hyaluronan, however, resulted in a remarkable reduction in both inherent and acquired resistance to the tested drugs.

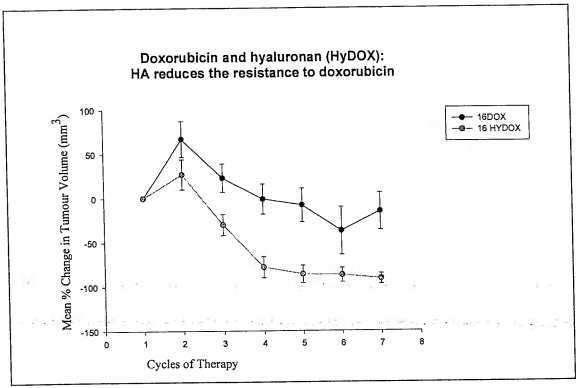
5-Fluorouracil and hyaluronan (HyFIVE):
HA reduces the resistance to 5-Fluorouracil

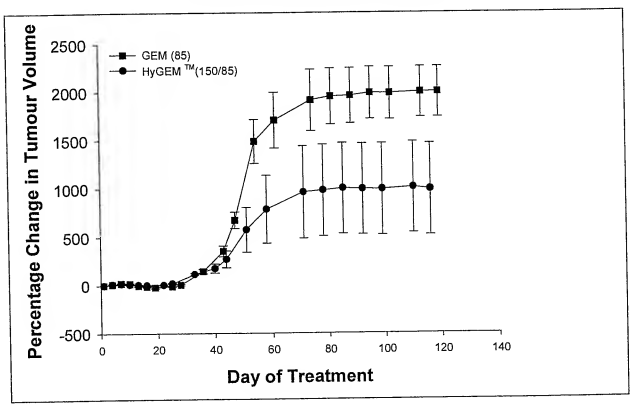


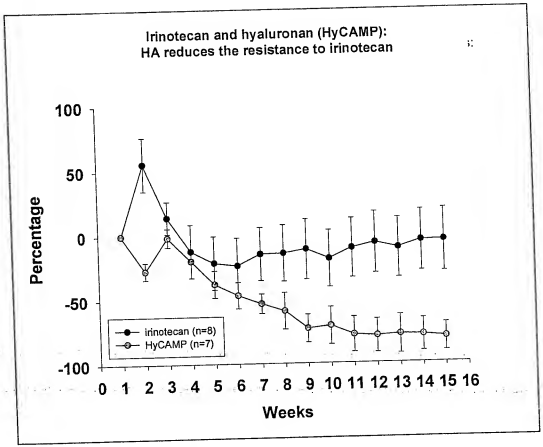
Methotrexate and hyaluronan (HyMET):
HA reduces the resistance to 5-methotrexate

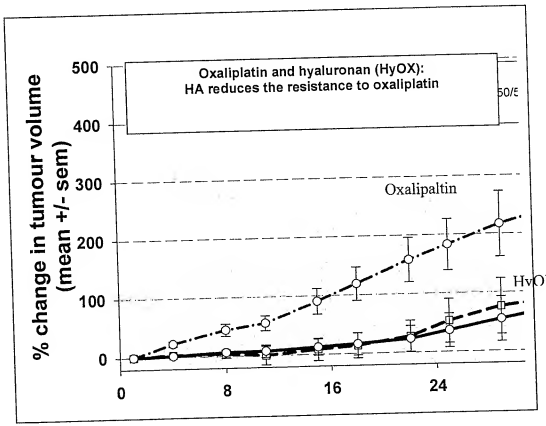
Figure 4
End-Point Tumour Volume
(expressed as % of change over treatment period)




Doxorubicin and hyaluronan (HyDOX):
HA reduces the resistance to doxorubicin
Mean % Change in Tumour Volume (mm3)
100
50
0
-50
-100
-150
0
1
2
3
4
5
6
7
8
Cycles of Therapy
16DOX
16 HYDOX


Gemcitabine and hyaluronan (HyGEM):
HA reduces the resistance to gemcitabine
Percentage Change in Tumour Volume
2500
2000
1500
1000
500
0
-500
0
20
40
60
80
100
120
140
Day of Treatment
GEM (85)
HyGEM ™(150/85)


Irinotecan and hyaluronan (HyCAMP):
HA reduces the resistance to irinotecan
Percentage
100
50
0
-50
-100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks
irinotecan (n=8)
HyCAMP (n=7)


Oxaliplatin and hyaluronan (HyOX):
HA reduces the resistance to oxaliplatin
% change in tumour volume
(mean +/- sem)
500
400
300
200
100
0
0
8
16
24
Oxalipaltin
HyOX